 5672

P.S. Steyn and R. Vleggaar (Eds), *Mycotoxins and Phycotoxins*
A Collection of Invited Papers Presented at the Sixth International
IUPAC Symposium on Mycotoxins and Phycotoxins, Pretoria,
Republic of South Africa, 22—25 July 1985.
1986 Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

KEYNOTE LECTURE

GLOBAL SIGNIFICANCE OF MYCOTOXINS

C.W. HESSELTINE
Northern Regional Research Center, Agricultural Research Service, US Department of Agriculture, Peoria, Illinois 61604

I am greatly honored by the invitation to present the keynote address at this IUPAC meeting on Mycotoxins and Phycotoxins. According to Webster's dictionary, a keynote address is "an address, as at a political convention, that presents the essential issues of interest to the assembly."

One way to take care of my commitment to present the "essential issues" would be to review what is known about mycotoxins, but this would be an impossible task. In addition to the hundreds of reviews and symposia, at least 20 books have been devoted to the subject and thousands of research papers have been published in the West alone since 1960. A large number of research papers and books also were published in Russia and Japan even before that date. Recently an interesting paper was published by Schoental (1984) entitled "Mycotoxins and the Bible", with the author citing evidence of mycotoxins in the ten plagues of Egypt described in the books of Exodus and Job. A second alternative method of presentation would be to select a small area of mycotoxin research and review only this. However, this would be of interest only to a few people.

Rather than review the past, it seemed interesting, appropriate, and perhaps useful to look into the future and suggest where we should be going in mycotoxin research and the control of mycotoxins in food and feed. For example, which mycotoxins will need the most attention in the future on a world-wide basis? Since this is an international meeting I have looked at the mycotoxin problems that remain to be investigated on a global scale. I suspect we have only seen the tip of the iceberg when it comes to mold toxins. The questions that need to be asked are: What are the most important mycotoxins? What commodities are the most likely to contain mycotoxins? What types of research are most needed? What is the magnitude of the economic loss? A recent article in the New Scientist (1985) suggests that one quarter of farm commodities contains mycotoxins. What areas of mycotoxin research have been exhausted? Where is the mold problem located - in the field, in storage, or in both?

It would be presumptuous on my part to give answers to these questions based only on my experiences or the opinions of my associates. The only way I could see to get some opinion was to take a poll of research workers in various countries on these questions. No more than four people were polled in any one country; in many instances, only one was contacted. Selection was based on knowledge and expertise in the field. In countries where a large volume of research has been published more than one person was contacted. Selection was not based on any one discipline but included food scientists, chemists, microbiologists, and plant pathologists. In all, 30 countries were involved in the poll: Sweden, Sudan, Indonesia, USA, Poland, Hungary, Yugoslavia, China, Taiwan, Japan, Netherlands, France, Italy, Germany, South Africa, India, Jamaica, Spain, Israel, West Germany, Ireland, Denmark, Finland, New Zealand, Egypt, Pakistan, Gambia, Canada, Thailand, and Turkey. The response was nearly 100 percent; much greater than I expected. I assured each participant that no individual or country would be identified. At this time, I would like to thank each of the people who contributed to this survey.

The first question on the survey was to name the six most important myco-

TABLE 1
Mycotoxins ranked in order of their relative importance.

Mycotoxin	1	2	3
Aflatoxins	31	3	3
Ochratoxin	7	3	4
Trichothecenes	5	13	4
Zearalenone	2	6	7
Deoxynivalenol	2	3	3
Citrinin	1	1	1
Sterigmatocystin		2	2
Patulin			2
Cyclopiazonic acid			2
Nivalenol		1	
Stachybotrys toxin		1	
Diplodiatoxin			1
Skin-irritating factor			1
Penicillium toxin		1	
Ergot		1	1
Phomopsin	1		

Twelve replies did not give any second and third ranking and 4 replies did not give a third rank.

toxins. Almost everyone answered this question and the results are shown in Table 1.

All replies gave a number one important mycotoxin. The aflatoxins were lumped together and 31 replies indicated it as the most important toxin. In some instances, second and third rankings were not given. A few replies placed two mycotoxins as number one. In these instances, each was recorded as being number one. One surprise was the selection of ochratoxin as most important in seven replies. In second rank, the leader was trichothecenes by a wide margin.

The second question asked for the names of the six commodities with the most mycotoxin problems in the respondent's area or country. These results are summarized in Table 2 and Table 3. Table 2 shows the commodities in which at least four replies ranked the item. Not all replies listed six commodities. In some instances, there was some uncertainty as to whether they represented the same or different crops. For example, beans were mentioned three times and soybeans only once. Were the listed beans soybeans or some other legume? Table 3 shows, without ranking, products that were listed only one to three times. A statement made repeatedly was that an imported commodity was a problem. This was noted in 13 instances, for corn (3), tree nuts (5), peanuts (2), feed (2), and milk (1).

The question was asked where the respondents thought the mold grew and produced the toxin - in the field, in storage, or in both locations. Those polled were overwhelmingly of the opinion that both were important (32), eleven answered storage and four specified in the field. A number checked both storage and field and these were considered to be both. Three did not respond to the questions. One or more suggested that a further problem was time during shipment, presumably the time in transit in ships.

Obviously a more difficult question to answer was how much money mycotoxins were costing a particular region or country. Of course, it was pointed out that the cost would vary from year to year. In a paper published recently about this matter, the authors quote the UN Food and Agriculture Organization's estimate that 25 percent of the world's food crops are ruined by mycotoxins (Mannon and Johnson, 1985). They further state that 10-50 percent of the grain crops in Africa and the Far East are contaminated. The answers to this question about the money losses per year due to contamination of mycotoxins were unsatisfactory in that the great majority of people did not give a monetary estimate. Eight did not answer the question, and 27 replied they could not estimate the loss. In some instances, the losses were reported as percentage of crop. For example, one respondent said that 3-5 million tons of maize were lost, and swine production decreased by 30 percent in 1984.

TABLE 2
Commodities with mycotoxin problems

Commodity	Rank 1	2	3	4	5	6	Total
Peanuts	13	10	1	1	1		26
Maize (corn)	13	8	6	4	2		33
Feed	4	3		2		1	10
Wheat	4	3	4	3	2		16
Barley	3	2		2	1		8
Cottonseed		1		7			8
Tree nuts	1	1	4	3	1	1	11
Rice	1	1		1	1		4
Cereals	3	2	1	2	1		9
Milk			4			1	5
Cheese		3	1			1	5
Sorghum			2	1	1		4

Three respondents did not answer the question.

TABLE 3

Commodities with fewer mycotoxin problems

Sunflower	1	Beans	3
Copra	3	Rye	2
Grasses	3	Oats	2
Meat	3	Lupins	1
Chili peppers	1	Fermented food	1
Garlic	1	Soybeans	2
Silage	1	Dried cassava	1
Apples	3	Potatoes	2

On the other hand, another reply said there was zero loss from mycotoxins. Many respondents stated they did not know the loss from mycotoxins but that it was high. A summary of the replies is shown in Table 4.

TABLE 4

Cost of mycotoxins

No answer	8
Cannot estimate	28*
Zero	1
\$1-5,000,000	5
\$5,000,000-1,000,000,000	8

*5 replied the cost was too high.

TABLE 5

Most needed mycotoxin research

Rapid & improved analytical methods	30
Effect on humans and domestic animals	20
Detoxification	17
Ecology and mycology	16
Toxicology	14
Chemistry (need for international standards 3)	13
Surveys	9
Biosynthetic pathways	3

The last two questions of the poll dealt with the perception of the direction of research. The first asked what kinds of research were most needed. Replies were not ranked but if the research was mentioned three or more times it is listed in Table 5. Because there were so many different kinds of answers some responses were grouped together to make the data manageable.

The last question, the most difficult to analyze, asked for responses about the least needed research on mycotoxins. Replies were very different and could not be put into satisfactory groupings. Twenty-three people did not respond and ten identical replies were made by two or more people. These were:

Detoxification
Mycotoxins produced by Alternaria and Penicillium
Biosynthetic pathways (5 replies)
Effect of aflatoxin on animals
Toxicological studies
Biochemical studies
Complex analytical methodology
Breeding to obtain crops resistant to mycotoxins
Surveys of known mycotoxins, especially aflatoxin
Metabolism of known mycotoxins in animals and man

Among the more interesting replies, each mentioned by only one person were: Structure of nontoxic metabolites of fungi, bureaucratic administration, mycotoxins in silage, occurrence of ochratoxin in sun-dried products, and highly sophisticated research not applicable to farmers. Obviously, these replies indicated not only problems in different countries but also personal feelings.

LITERATURE CLASSIFICATION

Another way to look at the direction of worldwide research on mycotoxins would be to evaluate subjects of study reported in the current literature. For many years, the US Food and Drug Administration group involved in mycotoxins studies kept a computer file on the world literature concerning toxins produced by fungi and plants. This file was made available to me, and so it was possible to take the total literature information put into the computer in 1984 and classify references according to various topics. Of course, some papers would fall in two or more subject matter categories; in these cases the one that seemed most appropriate was counted. In some cases it was difficult to decide whether the paper belonged under toxicology or physiology. Decisions were made as to which subject seemed most appropriate; the type of journal in which the article was published also helped in making the decision. Chemistry and biochemistry were lumped together since the two could not be separated just from the title of the article.

A total of 908 articles were reviewed and 255 articles were excluded. Excluded papers dealt with algae and protozoa toxins, as well as those which were concerned with natural toxins produced by the plant. The results of this classification of research papers were then compared to those recorded in 1980 in order to see if there were trends. The results of this survey are shown in Table 6.

TABLE 6

Classification of articles on mycotoxins recorded during 1984 and 1980.

	1984		1980	
	Number	Percent	Number	Percent
Mycology and taxonomy	28	3.08	60	6.83
Ecology	40	4.40	27	3.07
Production of toxins	57	6.28	42	4.78
Survey	71	7.82	62	7.05
Analytical methods (chemistry)	71	7.82	105	11.95
Analytical methods (biological)	16	1.76	7	0.80
Chemistry and biochemistry	114	12.55	107	12.17
Toxicology	39	4.30	48	5.46
Physiological effects	106	11.67	88	10.01
Diseases in domestic animals	23	2.53	15	1.70
Diseases in fowl	7	0.77	1	0.11
Carcinogenic effects	29	3.19	45	5.12
Detoxification	20	2.20	17	1.93
Food safety	14	1.54	8	0.91
Reviews	18	1.98	37	4.21
Papers not on mycotoxins	255	28.08	210	23.89
Total	908		879	

All toxins produced by fungi, including mushrooms, were counted and classified. This information generally confirms what most of us had assumed. The leading areas of research were in chemical analytical methodology, chemistry and biochemistry, and the effect of various toxins on the physiology of animals. Perhaps a little surprising was the number of papers on ecology.

I would like now to discuss three mycotoxin problems which are of the most concern on a worldwide basis. These problems are general in nature and do not refer to any one mycotoxin or any one commodity or to any one region of the world.

First, I would like to put mycotoxins in the context of the world food situation. As you know, there is worldwide concern about the world food supply both now and for the future. Research is striving to increase the quantity and quality of the food supply on a global basis. We know a great deal about animal nutrition; the importance of vitamins, the proper balance of amino acids, trace elements, and the prevention of disease with drugs, vaccines, and sanitation. What has not been taken into account until recently is the effect of inhibition of animal growth by low levels of natural toxicants, especially mycotoxins. For example, let us look at the broiler industry in the United States; it is highly developed and efficient in meat production because of agricultural research. Papers have appeared that demonstrate that mycotoxins are the cause of some problems in the broiler industry. This industry produced 17,037,999,000 pounds of broiler chickens in 1983, according to USDA's Agricultural Statistics for 1984 (United States Department Agricultural Statistics 1984). Let us assume that the weight gain was reduced only 3% due to low levels of mycotoxins in the feed. The figure of 3% is just a guess; we really don't know the magnitude of the loss. A 3 percent loss would be 511,139,970 pounds of meat we could have had were all the effects of mycotoxins removed from the birds' diet. At 28 cents a pound, then, the loss annually amounts to more than 143 million dollars. We realize that complete elimination of this loss cannot be attained, but it is a problem important enough to be attacked not only in the US but throughout the world. The losses are almost certainly even higher in some other parts of the world than they are in the United States. Our problems are similar, because molds know no borders; the same fungus species produce aflatoxin in the United States as in India or Africa. A meeting such as this one sponsored by IUPAC gives an excellent forum for exchange of information on mycotoxins and on means to reduce losses caused by fungi that reduce our world food supply.

Secondly, not enough attention has been given to the effect of some mycotoxins on the immune system in animals and man. Research has established beyond a doubt that some fungal toxins, such as aflatoxin, cause the animal's immune system to be reduced or destroyed. When low levels of mycotoxins are ingested by an animal, and then the animal is challenged by an infective microorganism, the animal's immune defenses are reduced and death occurs; whereas control animals not receiving low levels of toxins survive. Richard et al. (1978) have reviewed the subject and they point out that various immune systems are affected. Thus, resistance to disease is lowered, acquired immunity is interfered with, antibody production and cellular immunity are changed, and mechanisms of defense such as phagocytosis are altered. For example, turkeys and chickens fed 0.26-0.5 p.p.m. of aflatoxin B_1 during or after immunization to Pasteurella multocida (fowl cholera) were found not protected from this disease when exposed to challenge. Birds fed a normal feed without aflatoxin were protected. However, not all mycotoxins affect the immune system and, in fact, aflatoxin appears not to affect some animal/infective agent systems. Obviously much more research is needed in this field in both domestic animals and humans including an examination of the behavior of a variety of the more important mycotoxins. This problem of suppression of the immune system needs to be evaluated in relation to the official regulation of allowances of low levels of mycotoxins in animal feeds. Although the allowable levels of a mycotoxin may not appear to have any effect on the animal, indirectly they may lead to more infections by microorganisms because of the impaired immune protection systems.

A third area of concern is the importance of mycotoxins in human health. Illness in humans caused by mycotoxins may be a larger problem than anyone realizes. This is especially true in countries where foods are often in short supply and poor quality moldy foodstuffs have to be used to avoid starvation.

One needs to recognize that food poisonings caused by bacteria and molds generally differ in the time that elapses before the poisoning effects are felt. Bacterial poisoning is typically quick and violent. Enterotoxin produced by Staphylococcus makes the patient ill within a few hours and the onset is often violent, with vomiting and acute feelings of illness. On the other hand, fungal toxins do not typically produce rapid and acute illness. Usually longer periods of time elapse before illness is recognized, unless a huge amount of mycotoxin is consumed. This is usually recognized by hemorrhages in one or more internal organs.

A fact often overlooked is that mycotoxins were first recognized because of poisoning in humans rather than animals (Hesseltine, 1979). Toxins produced by mushrooms such as Amanita were known in ancient times, as well as ergot poisoning. These toxins are exceptions to the rule that fungus toxins are slower acting. In recent times several diseases in man were determined to be caused by mold products. These include dermatosis from psoralens in celery in US; alimentary toxic aleukia in Russia, poisoning caused by eating yellow rice in the Orient; vomitoxin; Phoma sorghina causing Onyalai disease in Africa; acute aflatoxin toxicity in corn in India; and the red mold scab in the Orient in wheat and barley (Akakobibyo disease). In addition to these proven poisonings of man by molds, a number of diseases have been suggested as being caused by mycotoxins. These include Balkan nephropathy, Reye's syndrome, Kashin-Beck in Siberia, epidemic polyuria in India, dendrodochium in Russia and acute cardiac type of beriberi in Japan.

A number of chronic diseases in humans occur where the causative agent is not known. Most likely some of these are caused by the ingestion of mycotoxins or a combination of mycotoxins and other toxins or infective microbial agents. Especially suspect would be illnesses in areas where moldy food products occur. However, the fact the food was made from plants infected with plant pathogens should not be overlooked even though no mold grew on the harvested crop. The toxin might have been produced in growing plants before harvest.

The third portion of my lecture is an enumeration and discussion of questions in mycotoxin research that have not been answered or are in great need of further study. Some are my own ideas whereas others are a result of reading the literature and of informal discussions with mycotoxin researchers. For convenience, I have divided the areas of needed research into the following subjects: mycology, ecology, surveys, analytical methods, chemistry and biochemistry, toxicology, diseases of animals, physiology, detoxification, and economics.

I. MYCOLOGY AND TAXONOMY OF FUNGI

All commodities, unless they are processed with heat or chemicals are inhabited by a mixed mold flora. For example, wheat flour always contains a small but regular population of Aspergillus candidus. In a few commodities, the regular flora has been determined not just for one year but also the variation for several years. This, then, gives a base line as to what the regular flora is in each commodity. This flora determination has been done for some of the world's major cereals. However, many other products exist where the normal flora either is not known or is known only for a restricted area. We need more studies of this nature on the flora of good quality products. This information then tells one which molds need to be examined for possible mycotoxins in each commodity. Unusual numbers of the normal flora or molds not typically associated with the usual flora, indicates that the product has been mishandled and needs to be viewed with suspicion.

There is no need to look for toxins in many fungi that never grow in our food and feed supply. Research should be concentrated on those fungi that either regularly inhabit the commodity or sporadically develop when the product is mishandled. Thus, it is a waste of time to look for toxins in Absidia glauca, a strictly saprophytic mold in soil, as compared to Absidia ramosa, a regular inhabitant of cereal grain. No toxin has been identified for this latter species, but it is known to induce infections in animals. Alternaria is seldom found in corn unless it is grossly mishandled, but examination of grain sorghum indicates that Alternaria is almost always present. Therefore, when examining sorghum for toxins, one should certainly evaluate the various toxins produced by this genus.

Basic studies are needed to establish the normal mycoflora of each commodity, not just in one year or in one geographical location.

Since Penicillium is such an abundant fungus in cereals this genus should be systematically investigated for toxins. I suggest that 10 strains of each species should be grown on two or three substrates such as corn, rice, and soybeans, and then checked for toxin activity in chickens and rats. In the drug industry's screening for antibiotics, many Penicillium isolates were found to be toxic to test animals, but usually these data were not published.

Taxonomic research should be directed at problems of identification of toxin-producing fungi. For example, a few years ago a study was made by Miss Dorothy Fennell and associates of the Penicillim viridicatum isolates. In P. viridicatum three distinct taxonomic groups were separated on the basis of their toxins: group 1 produced no toxin; group 2 produced citrinin and ochratoxin together or singly and group 3 produced only ochratoxin in 9 out of 13 isolates. There were slight but significant morphological differences as well as differences in rate of growth, colony color, type of growth, odor, and source of isolates. Group 3 came from meat products, whereas groups 1 and 2 came from wheat, peanuts, beans, and corn. Further study might have demonstrated that each type had consistent differences and the three groups actually represented three species.

Fusarium moniliforme is a common mold in corn. Studies in the US have demonstrated mutagenic metabolites in strains of this fungus. Research needs to be directed to what percentage of strains produces toxins and to just what components the mold uses when it grows in corn, as well as to what byproducts it produces.

In only a relatively few fungi have the optimal conditions been determined for mycotoxin formation, that is medium, time, temperature, and physical nature of the substrate.

Studies should be made on the viability of the mold in the commodity, as well as the stability of its mycotoxin. To be of any significance, the study must be done with the mold grown in the commodity not on laboratory media.

A method is needed to determine the amount of living and dead mycelium in the substrate. Plate counts are of no value and, although considerable work has been directed to this need, no satisfactory method is yet available for a mixed mold flora.

Study needs to be made of why certain molds, especially Fusarium, lose their ability to give high levels of toxin. Presumably, there are ways of inducing poor producers to increase their mycotoxin yield.

What role do virus-infested strains play in mycotoxin production? Are good toxin producers virus-infected or virus-free?

II. ECOLOGY

By ecology I am referring to the role the toxin-producing fungi play in mycotoxin formation. Fungal ecology deals with the mutual relations between organisms and their environment.

Further study needs to be done on finding the regions of the world where specific mycotoxin fungi occur and where their mycotoxins are regularly found. For instance, we know that corn grown in the tropics or semitropics is regularly contaminated with aflatoxins.

Study needs to be made of plant material infected with plant pathogens for mycotoxins. Instances are known where this occurs such as ergot and slaframine. One wonders about the cereal smuts, rusts, blights and root rots.

Investigations should be made on how the reservoirs of spores are preserved between growing seasons. Are they in the soil, on debris or in storage bins?

How does the seed become infected in the field or in storage? Are wounds required or, if not, then how does the fungus germ tube enter the plant. We know that some fungi can enter the plant through undamaged surfaces. Much of this work will have to be conducted in controlled environments and, therefore, the research will be expensive. One cannot help but believe that insects play an important role both in transporting the inoculum and in producing injury to the plant, especially to seed in the field.

An exciting field of research now emerging involves the interaction between the mycotoxin-producing fungus and other molds growing in association with it. Several years ago we made a study of a natural contamination of stored corn in which both zearalenone and aflatoxin were present. Even though kernels were attached to each other by mycelium, upon analysis individual kernels were found to contain only zearalenone or aflatoxin, but never both.

Breeding host resistance to a mycotoxin-producing fungus is possible for those that are true pathogens, but breeding resistance in plants to saprophytes seems more difficult and the results thus far have not been promising. Probably the only resistance of any importance would be the establishment of resistance barriers such as epidermal cells containing a mold inhibitor.

The movement of mycotoxins from one part of the plant to another: aflatoxin appears not to migrate in plants, but what about other toxins?

Optimal conditions for growth and toxin production are known for a few fungi but there are many others where these are not known. Likewise, we know little about conditions that inhibit mycotoxin production. For aflatoxin formation, we know that high temperature or high moisture levels adversely affect production.

Many of our studies are made under controlled conditions that remain constant during the experiment. Still, in nature, there are daily changes in temperature, available moisture, and growth of competitors.

A great deal more research needs to be done on the location of the mycotoxin in the plant. One level is in plant parts - seeds, leaves, stem, roots. A second level is the amount of mycotoxin in the various cellular tissues, for instance, corn germ. We know from work on peanuts that there is a varied distribution pattern of aflatoxin within a single seed.

Does the invasion of one fungus, for example into a corn kernel, make it easier for a mycotoxin producer to enter and produce toxin?

It is well established that some fungi are geographically restricted to certain parts of the world. Are any of these important in their local area as toxin producers?

III. SURVEYS

Survey for various mycotoxins depend upon accurate, dependable analytical methods. The lack of a good assay is probably the major cause of our ignorance on the natural occurrence of most mycotoxins. Surveys are important because they indicate the magnitude of the problem in a commodity and as well as what geographical areas are important.

Chemical methods used to assay the product should be accompanied by a mycoflora survey. A classical paper published recently told of sterigmatocystin found in a batch of cattle feed that caused feed poisoning (Vesonder and Horn, 1985). From this feed, sterigmatocystin was isolated; Aspergillus versicolor accounted for 28-60% of the mold colonies in the feed. Nine isolates of this species were selected at random from the feed and all produced sterigmatocystin.

For other mycotoxins besides aflatoxin, the sample size and the number of samples collected need to be determined statistically.

Research needs to be stepped up on the use of ELISA and monoclonal antibody techniques, especially for use in mixed feeds that become a problem with chemical methods because of (1) the varying types of materials involved, and (2) the changing costs of ingredients from day to day.

Care needs to be taken in deciding to do a particular survey. Certainly there needs to be a good indication that the mycotoxin being evaluated causes a real problem and occurs fairly often. Surveys, when done properly, are costly and time consuming and need to be carefully planned to give the most information.

IV. ANALYTICAL METHODS

Probably the greatest need for research on analytical methods is the development of a quick, cheap, simple method of indicating the presence of a specific mycotoxin. The black light method for detection of aflatoxin is the type of assay I'm referring to. A negative black light test on a properly collected, representative sample indicates that there is no danger. A positive black light test does not say that aflatoxin is present, but it does indicate that a more definitive assay, such as the minicolumn, should be run. We have no such method for other important mycotoxins such as zearalenone, ochratoxin and others.

Analytical methods should be developed only for those mycotoxins that are (1) known to occur naturally and (2) for those mycotoxins in which the producing fungus is known to occur in agricultural commodities on a regular basis.

Considerable progress has been made in developing multiple assays - assays that can be used to detect several mycotoxins at the same time. More work needs to be done on this research to increase the sensitivity and simplicity of the assay. Multiple assays should be based on mycotoxins which are likely to occur together; for instance, ochratoxin, citrinin, and penicillic acid are likely to be found in the same places because the optimal growth conditions of the penicillia that produce these toxins are similar.

As already mentioned, we need to do more work developing monoclonal antibodies and ELISA methods.

Some work has been done on the development of micromethods of analysis where very small quantities of material are available, such as parts of kernels, or of mycotoxins in or on fungal spores.

Proper collection of statistically size samples and sample preparation needs more attention. For example, should the same size (weight) sample of corn and wheat be rèquired to give meaningful results? Many fewer seeds are present in a 50 lb. sample of corn than in a 50 lb. sample of wheat.

V. TOXICOLOGY

Since toxicology is not my field of expertise, I have only a few suggestions. It appears that a great amount of research on the more common mycotoxins is already in the literature.

More studies appear to be needed on the effect of two or more mycotoxins being consumed at the same time by an animal.

More information is needed on where and how aflatoxin M_1 is made in the animal from aflatoxin B_1.

A new effect of mycotoxins should be examined as to whether or not the consumption of low levels of mycotoxins such as T-2 affect the behavior patterns in humans. Schoental (1985) this year suggested that Fusarium toxins may well produce psychiatric disorders.

The role of ochratoxin in Balkan nephropathy needs a great deal of research and could represent a model for study of other chronic diseases caused by mycotoxins in food consumed by man.

We need to do more research on the hazards of inhalation of dust containing mycotoxins and spores by persons handling moldy commodities such as grain and hay. Is there any relation between the allergies to mold spores and their toxin content?

VI. MYCOTOXICOSIS IN ANIMALS

Last year a conference sponsored by the UJNR Toxic Microorganisms Panel was devoted to the topic of diagnosis of mycotoxicosis in domesticated animals. The papers presented at that meeting will be published this year. Some 17 problem areas were identified where more research was needed in the study of mycotoxicosis:

The field of immunology has been neglected as a quick and accurate assay for mycotoxins, both in feed and animal tissues and excretion products. These methods, widely used in detection of bacterial toxins, were not developed for mycotoxins because the early work on detection procedures was based upon chemical and animal assays. We are now seeing the development of monoclonal antibodies, which should be especially useful in detecting toxin in mixed feeds where the ingredients may be constantly changed due to economics. Monoclonal antibodies are now available for aflatoxin and T-2 toxin.

More research needs to be done on known mycotoxins in other animal species. The effect of zearalenone on swine has been well documented but what is its effect on other animals?

Much more animal research needs to be done on the interaction of two or more mycotoxins. For instance, species of Penicillium and Aspergillus often grow together and one would expect to find their toxins together. What is the effect of this mixture on the animal?

In some instances toxins are produced by fungi in the roots of plants and can be transferred in an active state to the part of the plant eaten. How widespread is this? Some of the fungi known to produce toxins such as Fusarium and Trichoderma are well-known pathogens of the roots of domestic crops.

We are well aware that when aflatoxin is consumed by dairy cattle, aflatoxin M_1 will appear in the milk. But what about other mycotoxins such as zearalenone?

It is well established that there is varying resistance to aflatoxin in some varieties of chickens, and this is also true of other animals. Can resistance be bred into other species and breeds to protect the animal from a specific mycotoxin that cannot be economically excluded from the diet of the species. This may be especially important in some third world countries that depend a great deal on a specific animal for much of their protein and still have to depend on feeding contaminated feed.

A special problem exists in corn used as feed because Fusarium moniliforme occurs so regularly in it. Recent South African reports indicate that some strains of this fungus can cause tumors in rats and produce fusarin C, a mutagen. For example, rats fed a diet of 4% corn on which F. moniliforme had grown produced malignant tumors in 17 of 20 rats (Marasas et al., 1984). Corn is a special problem because it is so often infected with this fungus on a worldwide basis. What is needed is a determination of just how much corn is contaminated with this fungus, what the fungus uses in the corn for growth, and how widespread are toxins produced in corn in nature.

Suppression of the immune systems in animals is known to occur from aflatoxin and one or two other toxins have been examined for their effect on immune systems. Study needs to be broadened to other important mycotoxins such as vomitoxin, zearalenone, etc. The questions needed to be answered are whether all or most mycotoxins affect the immune systems and at what levels? How long does the impairment of any of the immune systems last? What is the effect of two or more mycotoxins ingested at the same time?

Not enough research has been done on the problem of detecting mycotoxins in mixed animal feeds. These feeds constantly vary because of the modification of the ingredients due to availability and cost.

Not much is known about what to do when animals become sick from mycotoxins except to get rid of the contaminated feed. What does the veterinarian tell the farmer whose herd is sick and his livelihood is threatened?

VII. DETOXIFICATION

Detoxification of aflatoxin with ammonia is the only satisfactory chemical method for detoxification of any mycotoxin. Methods need to be developed for the destruction of the more important mycotoxins but this requires a process that is (1) effective, (2) leaves no toxic residues, (3) is inexpensive, and (4) can be used by personnel with little training.

Little or no work has been done on the manner in which microorganisms degrade mycotoxins in nature. Hardly anything is known about the compounds produced when the mycotoxin is broken down.

The possibility should be explored on the use of harmless microorganisms which might be allowed to develop on a product that protects it from the development of other molds. This method has been used to preserve fish in the Orient so that no preservative or refrigeration is required. A process used by some rodents to protect their seed caches depends on controlled mold growth on their grain supply in their underground storage chambers.

Storage of cereals in storage bins in which anaerobic conditions are maintained has not received adequate study. This approach is now being used in some places commercially effective because most fungi will not grow under anaerobic conditions. This approach also is effective in controlling storage insects.

Another high priority should be engineering research on each of our major crops to learn how to reduce breakage damage to crops during harvest, drying, shipping, and storage. Broken seeds and fruits encourage the entrance of molds into the product in shipment and in storage. It is better to keep mycotoxins out of our food and feed than to remove them after the food and feed have been contaminated.

Genetic studies on corn, peanuts, wheat, and other major food crops need to be done to develop varieties resistant to insects and resistant to plant pathogens which are producers of mycotoxins.

Although mechanically separating aflatoxin-containing kernels from non-aflatoxin kernels has not succeeded, this method should be investigated for grain infected with Fusarium and other fungi that typically produce chaffy light kernels.

The blending of contaminated and clean grain is done rather widely, and in some places this is necessary because of the scarcity of feed. More research is needed on this process to determine the safety of the blended feed.

VIII. CHEMISTRY AND BIOCHEMISTRY

Research topics in chemistry have already been discussed in several other places in this paper, so the remaining number of future needs are not many.

Mycotoxins have a variety of effects on the animal, so it seems it would be worthwhile to study any mycotoxin or its derivatives to discover useful compounds. Zearalenol is now used as a growth promoter in beef cattle. According to Professor E. Schantz of the Food Research Institute, University of Wisconsin, botulinum toxin is now used in the treatment of certain eye diseases by utilizing the paralysis effect of the botulinum toxin.

Undoubtedly there are many compounds to be characterized and their structure determined. Purely basic studies should be made of molds commonly found in food and feed that show varying degrees of toxicity. This offers a good field for academic research.

Although the biosynthesis of the aflatoxins has been well worked out, much research is needed on the biosynthesis of other major mycotoxins, including the biosynthesis of the trichothecenes.

Intermediate compounds in the biosynthesis of the major mycotoxins need to be elucidated as to their chemistry and their biological effect. Enzymes involved in the biosynthesis of the naturally-occurring mycotoxins need to be isolated and characterized.

IX. ECONOMICS

No treatment of future research on mycotoxins would be complete without a discussion of economics.

We badly need information on the dollar losses for various commodities due to the growth of molds. These data are needed by country for each important commodity.

Secondly, we need to know the losses due to contamination with mycotoxins. That is, how much of the commodity is downgraded, thereby causing an economic loss. Or is the toxin-containing material in such bad shape it is a complete loss except for use as fuel or fertilizer. This information is of paramount importance in deciding the direction of research. From the research survey reported earlier in this report, the question on economic losses due to mycotoxins was the question that brought the fewest replies; even these were mostly guesses. Recently the UJNR Panel on Toxic Microorganisms published a book entitled "Control of the Microbial Contamination of Foods and Feeds in International Trade", and chapters dealing with the matter of losses in international trade raised more questions than answers (Kurata and Hesseltine, 1982).

The losses in weight gain, reproductive failures, death, and poor grade quality of animals fed contaminated feed are really unknown. I have estimated that there are as many as one thousand poisonings due to zearalenone to each one that is recognized and reported.

A second indirect loss is the amount of human illness, suffering, and death caused directly or indirectly by mycotoxins.

Where do the losses occur - in the field or during shipment or storage? I tried to find data on international shipment losses from marine insurers and government sources but I ran into a stone wall. Either the information does not exist or the information is kept secret.

Much work has been done on detoxification of aflatoxin in peanuts, cottonseed, and corn. What have been the savings? In a new process for detoxification of another mycotoxin, what are the savings? Would it be better to destroy the product or blend it off with good material to a level that would be harmless?

How do commodity losses caused by fungi compare with insect losses? The losses in strawberries must be much greater from fungi, but how about the cereals where storage insects are an enormous problem.

Farmers, shippers, and manufacturers need to be better educated in the prevention of mold growth. I remember visiting a company plant recently that had a recurring microbial spoilage problem. Although the manager did a thorough job of washing and cleaning both the machinery and floor, the problem still persisted. At one end of the plant was a large industrial fan that sucked dust from a dirt parking lot and dump into the plant. Besides the constant blast of air there was only a coarse filter that removed only rocks and cinders. The manager was quite unaware that his problem was coming through the air from the outside; the problem was easily remedied by placing the fan on the roof and installing adequate filters.

As scientists and administrators we need to evaluate how much equipment, manpower, and money needs to be allotted for research on each mycotoxin in our region.

This concludes a look into the future needs for mycotoxin research. Some of you, I'm sure, can add more important research that needs to be done and some of you may not agree with some of my suggestions. However, I hope that this talk will at least cause you to think in broader terms about mycotoxins and, perhaps, influence your thinking about your future directions of research.

Finally, I would like to thank IUPAC for inviting me to give this lecture and for their financial support. Also, I would like to thank the local organizing committee for assisting me in many ways.

REFERENCES

Hesseltine, C.W. 1979. In Interactions of Mycotoxin in Animal Products, Nat. Acad. Sci., Washington, DC, pp. 3-18.
Kurata, H., and Hesseltine, C.W. 1982. Control of the Microbial Contamination of Foods and Feeds in International Trade: Microbial Standards and Specifications, Saikon, Tokyo.
Mannon, J. and Johnson, E. 1985. New Scientist, 105: 12-16.
Marasas, W.F.O., Kriek, N.P.J., Fincham, J.E. and van Rensburg, S.I. 1984. Int. J. Cancer, 34: 383-387.
Richard, R.L., Thurston, J.R. and Pier, A.C. 1978. Toxins: Animal, Plant and Microbial. Pergamon Press, Oxford, pp. 801-817.
Schoental, R. 1984. Perspect. Biol. Med. 28: 117-120.
Schoental, R. 1985. Brit. J. Psychiatry, 146: 115-119.
United States Department Agricultural Statistics 1984. US Gov. Print. Office, Washington, DC, p. 360.
Vesonder, R.F. and Horn, B.W. 1985. Appl. Environ. Microbiol., 49: 234-235.